# Directing Traffic into the Future

## Find Your Goddess

**Bruno Antonny**
CNRS, Université Nice Sophia Antipolis

Meetings in the Schekman lab and 10 years later in the Rothman lab were intimidating: I had difficulty in learning the list of *SEC* genes and SNARE proteins that other lab members used there as an *esperanto*. However, I remember two extraordinary scientists who both cherish the same goddess: a small membrane sphere, transiently covered by a coat hiding fusogenic proteins. Through different strategies—genetics versus cell biology—to uncover what is necessary in a vesicle, they converged on biochemical reconstitutions to show what is sufficient.

For those who find the journey of a vesicle less inspiring in 2013, here's some advice. Take a close look at Figure 4 from the Jahn group paper "Molecular Anatomy of a Trafficking Membrane Organelle." The hundreds of lipids and proteins constituting the synaptic vesicle might trigger some dreams. Discuss with physicists and chemists: exotic words like "tension" or "packing" might be helpful. Work with people (with microscopes) who can help you really see membranes (as Schekman and Rothman did with Lelio Orci). Begin your own revolution by admiring membrane structures with a different charm than vesicles (have you heard about nucleoplasmic reticulum? transcellular tunnels?). Biological membranes resist simplification, and the 2013 Nobel Prize should encourage us to continue studying them.

## Sorting through Development

**Jon Audhya**
University of Wisconsin-Madison

The seminal contributions made by the three 2013 Nobel Laureates uncovered intricate intracellular communication pathways that enable information exchange between distinct membrane-enclosed environments. With the key factors in membrane fission and fusion revealed, we must now turn our attention to how their functions are regulated and appropriately integrated during development. Our laboratory takes advantage of simple nematode models, wherein we explore the function of conserved membrane transport systems during different stages of embryogenesis in vivo, whereas others have pioneered the use of *Drosophila*, zebrafish, and mouse models. We must also balance the dissection of fundamental regulatory mechanisms with approaches that can translate this knowledge into a better understanding of human development.

It is now clear that relatively subtle disturbances in membrane trafficking pathways, including mutations in the vesicle transport machinery identified originally by the Laureates, can underlie a range of debilitating ailments, including neurodegeneration, diabetes, and cancer. Recent advances in stem cell technology have opened up exciting possibilities to explore the roles of trafficking molecules using physiologically relevant cell types differentiated in culture. A close relationship between model organism- and stem-cell-based approaches will be instrumental in defining the mechanisms by which intracellular membrane transport contributes to tissue homeostasis.

## *SEC*s, Spines, and *VPS*

**Michel Bagnat**
Duke University School of Medicine

More than 30 years ago, Schekman and Novick isolated a set of yeast *SEC* genes and established the order in which they function, thus defining critical steps along the secretory pathway. Subsequent work by Emr and Stevens (both Schekman's trainees) uncovered the vacuolar protein sorting (*VPS*) genes controlling biosynthetic trafficking to the yeast vacuole.

My work on morphogenesis in zebrafish is deeply influenced by the yeast paradigm. When studying a complex process such as single lumen formation, we try to define distinct morphogenetic events by identifying mutations that block them, much like the *sec* mutants helped define the secretory pathway. We also find that *VPS* genes control the biogenesis and function of notochord vacuoles—fluid-filled organelles—during embryonic zebrafish axis elongation and spine morphogenesis. We keep returning to the original yeast papers for inspiration and to come up with new experiments to elucidate the cellular processes underlying notochord and spine formation.

*SEC* and *VPS* genes play specific roles during organogenesis across species, and understanding how the core secretory machinery is regulated for the assembly of different structures is a key question in development. Similarly, uncovering how polarized secretion is controlled in vivo to facilitate tube formation and function is fundamental for understanding organ development.

## Beyond the Core

**Julia von Blume**
Max Planck Institute for Biochemistry

Thanks to the pioneering work by Rothman, Schekman, and Südhof, the path is now open for a number of new and old unaddressed issues in intracellular protein transport and secretion beyond the core pathway.

My introduction to the field came when I met Vivek Malhotra, who had worked with Jim Rothman. Vivek had purified COP-I vesicles and found the involvement of NSF in reactions that control cycles of membrane fusion during trafficking. Impressed by his creativity, imagination, and enthusiasm, I joined his lab as a post doc to work on the mechanism of secretory cargo sorting at the *trans*-Golgi network (TGN) and am continuing this in my own group.

We investigate a TGN cargo-sorting process dependent on actin and $Ca^{2+}$ but independent of receptors. Understanding it is essential to unraveling how key factors like neurotransmitters, growth factors, and hormones are released. How bulky cargoes like collagens that are too big to fit into a COP-I- or COP-II-like vesicle are secreted is another pressing question. If proteins cannot enter the conventional ER-Golgi secretion pathway, how are they released by cells?

To progress in mechanistic understanding in these areas, we need ultrafast microscopy and reagents to rapidly inactivate trafficking machinery components so that imaging in primary cell culture can be combined with traditional biochemical analysis to bring new insight.

## Under an Electron Microscope

**John A.G. Briggs**
European Molecular Biology Laboratory

We use electron microscopy (EM) to study enveloped viruses and coated vesicles. EM has been a key research tool in membrane trafficking since the start of the field. George Palade used it when first describing the secretory pathway. This work inspired Randy Schekman and Jim Rothman to identify and characterize the trafficking machinery that we now study in the lab. EM has since been widely applied: by cell biologists to study membrane morphologies and by structural biologists to reveal structures such as the clathrin cage. Thanks to the pace of technology development in microscopy and image analysis, I believe that EM still has a major role to play.

Two recent directions appear particularly relevant for the field. First, there are a growing number of methods for correlating fluorescence and EM images of the same sample. Such methods allow fluorescently tagged cellular components to be identified by EM; rare or dynamic trafficking events can thereby be located and imaged with ultrastructural detail. Second, cryoelectron tomography is being used to visualize cellular components in vitrified cells and in complex in vitro systems. Combined with image processing, this can generate 3D structures of coat proteins in their membrane-assembled state.

I am optimistic that EM can contribute to a structural view of membrane trafficking: How do the components assemble into large, dynamic, and adaptable protein coats, able to collect cargo and to reshape the membrane to bud vesicles?

## Trafficking through Immunity

**Claudio Giraudo**
University of Pennsylvania

Breakthrough discoveries in intracellular membrane trafficking over the last three decades have shaped our current view of how components are transported within cells. These processes allow cells not only to maintain their homeostasis but also to communicate with other cells and to defend from external aggressors. In particular, immune cells utilize different secretory pathways for releasing chemokines, cytokines, antibodies, or cytolytic proteins as an integral part of both innate and acquired immunity. An increasing number of life-threatening inflammatory conditions, immune deficiencies, autoimmune diseases, and cancers have been associated with deregulation of membrane trafficking in immune cells.

However, we are still far from fully understanding the protein machinery that specifically controls the release of each of these substances and how these machineries are orchestrated during the immune response. Providing mechanistic insights into the molecular defects underlying these disorders will require us to overcome some major hurdles. We must first develop novel in vivo tools to dynamically study these processes with sufficient resolution. In addition, refinement of current techniques for T cell genome editing using endonucleases will be necessary, as will finding methods or compounds to acutely inactivate protein function. Only then will we be in a position to effectively manipulate membrane trafficking as a powerful tool for targeted immunotherapies.

## Turning Wiring into Action

**Pascal S. Kaeser**
Harvard Medical School

Perhaps the most complex membrane trafficking machine is the presynaptic nerve terminal, where synaptic vesicles fuse with the plasma membrane within less than a millisecond of the incoming calcium signal. This year's Nobel Prize in Physiology or Medicine celebrates the discovery of the molecular machinery that enables such fast, calcium-triggered fusion. The findings also motivate important questions.

The brain uses striking diversity in its building blocks. The remarkable drawings of neurons by Cajal illustrated this heterogeneity at the cellular level more than 100 years ago. Is such diversity also found in the molecular composition of the presynaptic release apparatus across synapses? And, if it is, what is its importance?

Properties of release are synapse specific, and release changes as a function of activity. This indicates heterogeneity in the protein networks that control the core fusion machinery. The neuroscience community is currently making a push to map how neurons are connected and to identify activity dynamics in neural circuits. One key to relate these efforts with one another lies in the presynaptic trafficking apparatus: understanding how each nerve terminal is built for site-specific information processing within a circuit may help relate the circuit wiring diagram to its activity pattern. The groundbreaking work on vesicular cargo sets the stage to dissect the molecular diversity of circuit relays that transform connected neurons into dynamic networks.

## Targeting the Noncanonical

**Elizabeth Miller**
Columbia University

Pioneering studies by Rothman, Schekman, and Südhof created the canon for the molecular basis of vesicle formation and fusion that defines eukaryotic protein secretion. Yet noncanonical protein transport also plays important roles in human physiology, including lipid homeostasis, embryonic development, and the immune response. Understanding the mechanisms of unconventional secretion offers hope for a new wave of therapeutics that could target specialized secretion events while leaving the essential standard pathway intact.

Canonical coat scaffolds self-assemble to generate small spherical vesicles. Some noncanonical secretion simply tweaks this process by employing accessory proteins that presumably modify the standard carriers to permit traffic of large cargoes like procollagen. A second version of noncanonical secretion corresponds to vesicles that are seemingly made without the help of known coat scaffolds. Here, lipid modification and redistribution as well as the biophysical properties conferred by cargo proteins may play important roles. Finally, truly unconventional secretion involves completely vesicle-independent mechanisms, moving further away from the canon of coat-enforced secretion. Understanding how these diverse molecules traverse the membranes that separate them from their site of synthesis and the extracellular milieu remains an exciting challenge.

## Mechanism and Biophysics

**Karin Reinisch**
Yale School of Medicine

Nearly four decades ago, Albert Claude, Christian de Duve, and George Palade (who founded my department) received the Nobel Prize for their discovery of the secretory pathway and membrane trafficking. Since then our efforts have become increasingly focused on a mechanistic understanding of how membrane trafficking events take place, including not only which proteins participate but also how these proteins interact with one another and with membranes. The Nobel Prize this year to Jim Rothman (my chairman), Randy Schekman, and Thomas Südhof recognizes this trend.

The Nobel discoveries, 39 years ago and today, were made possible by the addition of new methodologies to the biological toolkit, including cell imaging and genetics. To dissect the detailed molecular interactions that underlie mechanism, we need to incorporate biophysical methods into experimental approaches. These methods include crystallography, single-particle EM, and nuclear magnetic resonance (NMR), and structural biologists (like me) are being integrated into the cell biology community. Nevertheless, we are not adept at studying many complexes important for membrane traffic because they are too flexible or transient (X-ray, EM) or large (NMR). Single-molecule methods and computational modeling will therefore become increasingly important. Developments in membrane biophysics should yield much-needed insights into membrane behavior. As the field further matures, we are seeing further diversification in what it means to be a ‘‘cell biologist.’’

## Trafficking Computes

**Ivo F. Sbalzarini**
Center of Systems Biology Dresden, Max Planck Institute of Molecular Cell Biology and Genetics

The functioning of cells is intimately linked to uptake, processing, and release of a multitude of signals. Cellular trafficking is key to this information processing, involving localized biochemistry in multiple dynamic compartments with still-elusive molecular and biophysical details. Concerted trafficking of chemical signals between compartments allows the cell to "compute," i.e., to exchange information and take decisions. But this type of computation is fundamentally different from that in human-made computers.

Cellular trafficking has thus inspired the theoretical concept of membrane computing, also named "P-Systems" after G. Păun. A membrane-computing system, like the trafficking system in cells, processes a multitude of signals in parallel and in a distributed manner. It consists of many membrane-bound compartments, within which signals are processed, and which are able to move, fuse, and split, hence communicating. This concurrent, stochastic processing is conceptually different from the sequential, deterministic working of electronic computers. Systems approaches such as P-Systems may thus help address questions so far unanswered: What can a cell compute? Does the cellular trafficking system have a defined state, or does it probe multiple states at once? Can we unravel the rules of its working by watching it with computational imaging methods? The pioneering work by the Nobel Laureates hence continues to inspire questions and progress across discipline boundaries.

## Systematic *SEC* Biology

**Maya Schuldiner**
Weizmann Institute of Science

The isolation and characterization of the *SEC* genes, partaking in secretion of budding yeast proteins, were game changers in cell biology. How could one line of research, in a simple model system, have such a profound effect on generations of biologists to come? First, Schekman's studies uncovered the universal underpinnings of the eukaryotic secretion machinery. But even more remarkable was that the discovered convergence between the yeast and mammalian machineries underscored the conserved nature of cellular functions, turning yeast into the best-studied model system for cell biology.

The idea to systematically characterize a pathway or process, which preceded its time by 30 years and now lies at the heart of the field of systems biology, is what most sparks my personal passion as a researcher. It is this aspect of Schekman's work that drives my lab's efforts today, as we use systematic screening approaches to uncover proteins important for a variety of organellar functions. But probably most importantly, the quantum leap that Schekman, Rothman, and Südhof made by taking the "black box" of secretion and turning it into something that we can understand and manipulate was magical. These scientists being awarded the Nobel Prize reminds us that we should never stop looking for that magic as we pioneer forward in still-uncharted scientific waters.

## New Tools for Old Questions

**Jingshi Shen**
University of Colorado at Boulder

The now-established core engines of vesicle trafficking are often controlled by networks of regulatory factors to adjust the rate and direction of membrane transport according to physiological demands. Such regulated vesicle trafficking plays central roles in nearly every aspect of human physiology. One prominent example is insulin-triggered exocytosis of the glucose transporter GLUT4, which maintains our body's glucose homeostasis. It poses tremendous challenges to dissect regulated trafficking pathways because they exist only in highly specialized cells and because each pathway engages a unique set of regulatory factors.

Fortunately, nowadays we are armed with panoply of new tools unimaginable just a few years ago. In particular, induced pluripotent stem cells (iPSCs) have been successfully programmed into a variety of cell types in which regulated vesicle trafficking can be probed using the rapidly evolving genome-editing tools. Even more excitingly, unbiased genetic screens are finally possible in mammalian cells with the isolation of multiple haploid cell lines including iPSCs. Genome-wide screens in these haploid cells hold great promise in systematically identifying the specialized machinery of regulated trafficking pathways integral to human physiology. Looking forward, I expect these revolutionary technologies, combined with existing biochemical and biophysical approaches, to pave the path for the next breakthroughs in vesicle trafficking research.

## Vesicle Identities in Motion

**Shigeo Takamori**
Doshisha University, Kyoto

Studies pioneered by this year's Nobel Prize winners have revealed the basic molecular underpinnings of eukaryotic membrane trafficking. In particular, SNARE proteins, the essential catalysts of membrane fusion, are divided into two categories: "v-SNAREs" sit on vesicles and "t-SNAREs" are the v-SNARE acceptors on the target membrane. Because the mammalian genome contains several dozens of SNARE genes, we can now envision that the proper SNARE pairs define individual transport pathways and, together with Rab proteins, guarantee the accurate delivery of transport vesicles and prevent "unwanted" fusion events. However, many vesicles such as synaptic vesicles contain large sets of SNAREs and Rabs implicated in both exocytosis and endosome fusion, indicating the involvement of more complex fusion events.

Fundamental questions still remain. How do SNARE proteins find their appropriate locations in the cell? How is unwanted (noncognate) SNARE complex formation prevented in living cells? After fusion, how can only v-SNAREs return back to their original location? Collectively, how can vesicle identities be maintained in motion?

Having witnessed the recent advances in superresolution imaging techniques and amenable fluorescent probes, it is foreseeable that efforts to develop better ways to track dynamic movements of fluorescent-tagged SNARE proteins and organelles simultaneously in living cells will be fundamental to solve some of these issues.

## How to Keep Synapses Alive

**Patrik Verstreken**
VIB, University of Leuven

How do neurons maintain reliable neurotransmission throughout their long life, when it can take over a month to transport fresh material from the soma to the nerve ending? This question is particularly relevant in the context of disease such as Parkinson's, in which processes such as defective mitochondrial turnover, synaptic endocytosis, or autophagy may poison synaptic vesicle trafficking by failing to remove dysfunctional organelles, proteins, and lipids that regulate synaptic vesicle transport.

While it seems conceivable that the disruption of such processes results in vesicle cycling defects and ultimately in synaptic demise, the underlying mechanisms are much less clear. How are dysfunctional synaptic proteins sorted? How are chaperones, autophagy, endosomes, and lysosomes implicated? And how do these processes impinge on specific aspects of the vesicle cycle?

These questions are only now being unraveled but are intimately connected to the organization of cellular transport systems. For example, synaptic endosomes act as sorting stations for dysfunctional synaptic vesicle proteins that should be degraded. Hence, activating endosomal traffic at nerve terminals improves synaptic function. Similarly, chaperones that protect SNAREs are key to maintain synaptic efficacy. In the future, we will need to address how specific vulnerable sites in the vesicle cycle contribute to neuronal loss and how mechanisms that promote synaptic activity can be exploited to keep synapses alive.

## The Stuff Dreams Are Made of

**Tobias Walther**
Yale School of Medicine

Scientists at their best illuminate important processes by reducing them to seemingly simple concepts and reactions, which in turn change the way we think.

After coming to Yale, I witnessed a moment of a Master of Science in action as Jim Rothman explained to students that all their dreams—along with all their other thought processes—depend on the extremely fast and faithful zippering of SNARE complexes mediating the release of neurotransmitters. Asked by a student how to choose a problem to study, Jim answered, "Tackle a universal problem where you cannot imagine how it works."

With their elegant contributions, recognized this year with the Nobel Prize, Jim Rothman, Randy Schekman, and Thomas Südhof have largely resolved one such universal problem: how membrane vesicles are formed and deliver cargo to the right place in the cell. But important challenges in membrane biology remain. The regulation and distribution of lipids, a universal energy currency and core membrane component, remains largely mysterious. Despite formidable progress in our understanding of the regulation of some species, such as cholesterol, the fundamentals of how lipids are sorted, stored, mobilized, and regulated are not understood. New technologies to manipulate and analyze cell components comprehensively, using genome editing or novel imaging and mass spectrometry approaches, promise breakthroughs. The understanding of the mechanisms we cannot yet imagine is just around the corner.